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Specific Induction of Fibronectin Binding Activity by Hemoglobin in *Candida albicans* Grown in Defined Media

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Fibronectin (FN) is a major component of host extracellular matrix that may play an important role in the initiation and dissemination of *Candida albicans* infections. Expression of FN binding requires growth of *C. albicans* blastoconidia in complex medium, and the regulation of FN receptor expression is poorly understood. We now demonstrate that hemoglobin is a potent and specific inducer of FN receptor expression and describe a defined medium supplemented with hemoglobin that greatly and stably enhances the binding activity of *C. albicans* for soluble FN. Enhancement of FN binding by hemoglobin in strain 44807 was concentration dependent and was maximal at 0.1% hemoglobin with 20- to 80-fold enhancement. The hemoglobin-induced FN binding to *C. albicans* was saturable, with a K_d of 2.7×10^{-8} M. Enhancement required growth of *C. albicans* in hemoglobin-containing medium, since simply exposing blastoconidia to hemoglobin in a nongrowing status did not enhance binding. Induction was reversible following removal of hemoglobin from the growth medium and not associated with germination. Inorganic or protein-bound iron was not sufficient for the induction, since other iron-containing proteins or inorganic iron salts were inactive. Growth in the simple medium yeast nitrogen base supplemented with hemoglobin increased cell adhesion to immobilized FN and to cultured monolayers of bovine corneal endothelial cells. These data suggest that hemoglobin may be an important regulator of FN binding activity in *C. albicans* and thus may play a role in its pathogenesis.

Candida albicans is an opportunistic pathogen of humans that causes a variety of diseases, from superficial candidiasis to deep-seated infections such as endocarditis and nephritis in immunocompromised hosts (6, 14, 27). Adhesion of *C. albicans* to host tissues, mediated through binding to various extracellular matrix proteins such as fibronectin (FN) and laminin, is correlated with pathogenicity (6, 17, 20, 27, 29, 30). In common with mammalian cells (5, 30, 36) and some pathogenic bacteria (3, 34), several domains of FN are recognized by *C. albicans*. The Arg-Gly-Asp motif and additional sites in the cell-binding domain and in the collagen- and fibrin-binding domains of FN can mediate binding in solution or adhesion of *C. albicans* to substrates (21–23, 27, 29). These interactions may be mediated by discrete receptors or a single promiscuous receptor. Several candidate receptors for FN have been identified on *C. albicans*, including homologs of mammalian integrins (16, 33) and 60- and 105-kDa glycoproteins (21).

Identification and characterization of these and novel receptors for FN have been limited by the variability of their expression in clinical isolates and laboratory strains. Our laboratory experience (27) and that of others (22) indicated that the ability of *C. albicans* to bind FN varies greatly between different lots of individual media and is influenced by growth density of yeast cells, growth temperature, and shear rate during growth. Moreover, FN binding is observed after growth in a complex medium, such as Sabouraud broth, but not in blastoconidia grown in defined medium.

In an attempt to stabilize the expression of a *C. albicans* FN receptor(s) in our laboratory, we tested the role of specific medium components in the induction of FN binding during growth in defined media. The purpose of this study was to develop a medium that would allow stable expression of FN binding activity in *C. albicans*. Among the supplements used, human hemoglobin was found to specifically enhance binding of FN to *C. albicans*. *Candida* cells grown in hemoglobin-containing medium demonstrated an increased adhesion to immobilized FN as well as to endothelial cells. Identification of hemoglobin as an inducer of FN receptors on *Candida* cells may facilitate further characterization of FN-mediated adhesion and its role in pathogenesis of *C. albicans* infections.

MATERIALS AND METHODS

Strains and growth conditions. *C. albicans* ATCC 44807 was used throughout this study unless otherwise specified. Its binding to FN has been well characterized in Sabouraud medium (27). Other *Candida* strains were collected from clinical patients from the NCI Pediatric Branch, Bethesda, Md. Cultures were grown routinely in freshly prepared Sabouraud broth for 20 h at 26°C on a rocking platform except where otherwise indicated. The stationary-phase blastoconidia were aliquoted and frozen at −70°C until used. For each experiment, the organisms were thawed, inoculated into 6 ml of specified medium with or without supplements, and incubated as described above for 20 to 48 h. Under the growth conditions with all media used, no germination was found upon microscopic examination.

Medium modification and supplements. Three commonly used media for fungal growth were assessed in terms of their influence on the FN binding ability in *C. albicans*. BBL modified Sabouraud broth (antibiotic medium 13) was purchased from Becton Dickinson Microbiology Systems (Cockeysville, Md.), yeast nitrogen base (YNB) was from Difco Laboratories (Detroit, Mich.), and Lee Buckley Campbell (LBC) broth was from the NIH Media Unit (Bethesda, Md.). Of these media, Sabouraud broth is a complex, undefinable medium whereas YNB and LBC contain only defined low-molecular-weight nutrients (1). A number of host proteins added to defined media were tested for their ability to influence the binding of FN to *C. albicans*. Blastoconidia were incubated in medium supplemented with 1 mg of human hemoglobin, horse heart myoglobin, human gamma globin, bovine serum albumin (BSA), human transferrin, α-casein from bovine milk, or human α-acid glycoprotein per ml and processed as described above. Optimal binding of *C. albicans* to FN induced by hemoglobin was obtained with YNB medium prepared at four times the normal concentration (1).

High- and low-molecular-weight components of Sabouraud broth, Peptamin, and Casitone (purchased from Difco Laboratories) were separated by dialysis against distilled water overnight at 4°C in dialysis tubing with a molecular weight cutoff of 10,000. These components were then lyophilized, reconstituted in water

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to the original volume, and sterilized by filtration. FN binding activity was determined in *Candida* cells grown in Sabouraud broth or high- or low-M_r components of Sabouraud medium. In addition, various supplements of either inorganic salts or proteins were added to simple medium, and their influence on *Candida* binding to FN was determined. All inorganic salts and proteins used in this study were purchased from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise indicated.

FN binding assay. FN was purified from frozen human plasma as described previously (27). Iodination of FN or other proteins (with a specific activity of 1 to 2 μCi/μg) was accomplished by using Iodogen (Pierce, Rockford, Ill.), and unbound iodine was removed by passage through a PD-10 column (27). In a typical binding assay, 2×10^6 *C. albicans* cells were exposed to ^{125}I-FN at a final concentration of 0.5 μg/ml in a total volume of 200 μl of Dulbecco's phosphate-buffered saline (DPBS) without $CaCl_2$ and $MgCl_2$ but with 0.1% BSA (pH 6.0) in a polypropylene tube and incubated for 3 h with shaking on a rotary plate at 160 rpm. The cell suspensions were transferred to microtubes, and blastoconidia were separated from unbound ^{125}I-FN by centrifugation through 100 μl of an oil mixture of dibutyl phthalate-dioctyl phthalate (2:1). Radioactive FN bound to the cell pellet was counted in a gamma counter (Packard Instrument Co., Downers Grove, Ill.).

Cell adhesion assay. Bovine corneal endothelial cells (26) were grown on eight-well glass Chamber Slides (Nunc, Inc., Naperville, Ill.) to confluence in Dulbecco's modified essential medium supplemented with 10% fetal calf serum, 1% glutamine, and both penicillin and streptomycin at 35°C. *C. albicans* cells were prepared in YNB medium with or without 0.1% hemoglobin as described above. For adhesion assays, endothelial cells on Chamber Slides were gently washed three times with DPBS (pH 7.2; without $CaCl_2$ and $MgCl_2$) and incubated with *C. albicans* in DPBS (2×10^6 CFU/ml) for 2 h at room temperature without shaking. Chamber Slides without bovine corneal endothelial cells served as a control for testing nonspecific adhesion by *C. albicans*. At the end of the incubation, unattached *Candida* cells were removed by gently washing three times with DPBS. Cells on the slides were fixed by incubation with 1% glutaraldehyde in DPBS and stained with DiffQuick (Baxter Scientific Products, Miami, Fla.). Adhesion was expressed as the number of *Candida* cells (excluding cell aggregates with more than 4 cells), per square millimeter, attached to either endothelial cells or immobilized FN. The latter was prepared by adding 200 μl of FN solution to each well of the Chamber Slides and incubating the slides at 4°C overnight.

RESULTS

Comparison of media in the induction of FN binding activity. Of the three media tested, *C. albicans* blastoconidia grown in Sabouraud broth for 20 to 48 h produced the highest FN binding activity (Fig. 1A). Sabouraud medium is a complex medium, whereas the LBC and YNB media are defined media. *C. albicans* grown in the high-M_r portion of Sabouraud broth demonstrated a higher activity in binding to FN than those grown in the low-M_r fraction (Fig. 1B), suggesting that the high-M_r components of Sabouraud medium are necessary for the expression of FN binding activity.

Peptamin and Casitone are the two complex ingredients contained in Sabouraud medium. Peptamin and Casitone were separated into high- and low-M_r components by dialysis, and their effects on the expression of binding to FN were also assessed in defined media. Growth of *C. albicans* in the high-M_r components of Peptamin increased binding to FN similar to that in the high-M_r components of Sabouraud broth, whereas growth in the high-M_r components of Casitone resulted in a smaller increase. In contrast, the low-M_r components of Sabouraud broth, Peptamin, or Casitone did not enhance FN binding activity (data not shown). These results indicated that high-M_r components of Peptamin played a major role in the higher FN binding activity of *C. albicans* grown in Sabouraud medium relative to those grown in LBC or YNB containing only low-M_r nutrients.

Hemoglobin enhancement of *Candida* FN binding activity. Peptamin is a proteolytic digest of animal tissues. Although the high-M_r components of Peptamin demonstrated an enhancement in FN binding to *C. albicans*, its contents are still far too complex to define. On the basis of the observation that supplementing simple media with high-M_r fractions of Peptamin moderately stimulated *Candida* binding to FN, several host proteins that *C. albicans* may encounter were tested for their influence on FN binding activity. Of the proteins tested, only hemoglobin (1 mg/ml) strongly enhanced FN binding. Gamma globulin, globin, and myoglobin demonstrated a moderate enhancement. However, no noticeable enhancement was observed for other proteins examined, including human α-acid glycoprotein, bovine α-casein, human holotransferrin and apotransferrin, and BSA (Fig. 2). Other than hemoglobin, myoglobin was the only iron-containing protein tested that slightly enhanced FN binding to *C. albicans*. However, this slight enhancement was not consistently observed. Binding of *Candida* cells grown in the presence or absence of hemoglobin to radiolabelled BSA or human α-acid glycoprotein did not demonstrate an enhancement similar to that of FN binding (Fig. 2, insert).

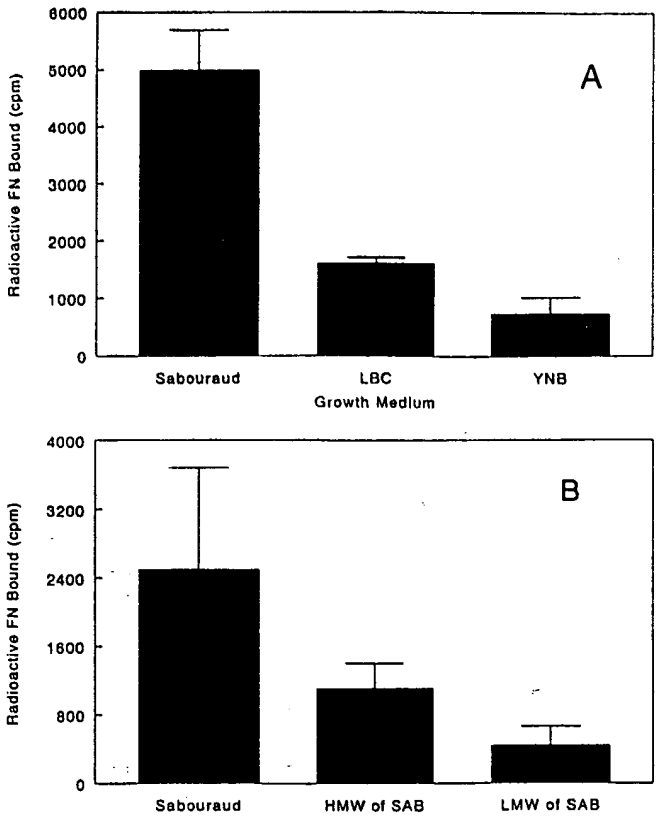

FIG. 1. Effect of growth medium on FN binding to *C. albicans*. *C. albicans* ATCC 44807 cells were inoculated into Sabouraud broth, LBC medium, or YNB medium (A) or into Sabouraud broth and its separated high- and low-molecular-weight components (HMW and LMW, respectively) from dialysis (B) and incubated overnight at room temperature on a rocking platform. FN binding activity from these cultures was assayed by incubating 2×10^6 *Candida* cells with ^{125}I-FN in DPBS in a total volume of 200 μl for 3 h at room temperature. The bound radioactivity was quantified as described in Materials and Methods, and the data are presented as means ± standard deviations ($n = 3$).

Hemoglobin is a tetrameric protein composed of globin subunits and heme groups. At a molar concentration equal to that of the iron contained in 0.1% hemoglobin, inorganic ferrous ion, protoporphyrin IX, and hemin were tested individually or in combination with globin supplemented in YNB medium. Although globin plus ferrous ion moderately enhanced binding, none of these media demonstrated an effect on FN binding comparable to that of hemoglobin (Fig. 3).

Characteristics of hemoglobin-induced FN binding in *C. albicans*. The addition of less than 0.01% hemoglobin to either LBC or YNB medium significantly enhanced the binding of FN

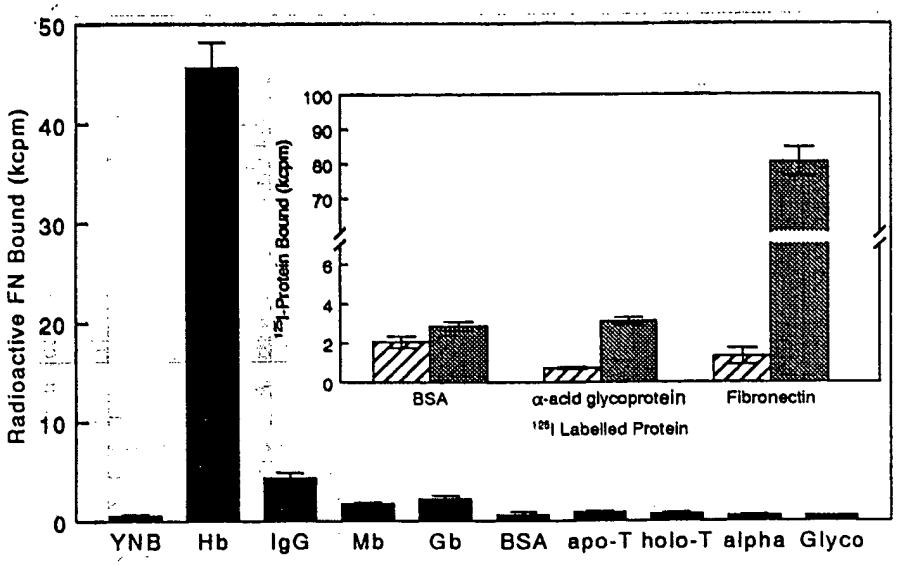

FIG. 2. Effect of protein supplements in YNB medium on FN binding to *C. albicans*. *Candida* cells were inoculated into 4× YNB simple medium or medium containing various protein supplements at a final concentration of 0.1% each and incubated at room temperature for 20 to 48 h with shaking on a rocking platform. FN binding activity was assessed as described in the text, and the data are presented as means ± standard deviations ($n = 3$). Abbreviations: Hb, human hemoglobin; IgG, human gamma globulins; Mb, horse heart myoglobin; Gb, human globin; apo-T, apotransferrin; holo-T, holotransferrin; alpha, α-casein from bovine milk; glyco, human α-acid glycoprotein. The insert shows the binding of radiolabelled BSA, human α-acid glycoprotein, and FN to *Candida* cells grown in 4× YNB medium with (shaded bar) or without (hatched bar) hemoglobin.

to *C. albicans*. A plateau was reached at the concentration of 0.1%, and the resultant binding activity enhanced by hemoglobin was higher in YNB than LBC medium. Fresh human hemoglobin prepared by hypotonic lysis of washed erythrocytes (31) was also tested and demonstrated an effect on enhancement of binding similar to that of commercial animal hemoglobin. No germination occurred when *C. albicans* was grown under such conditions. The enhanced FN binding to *Candida* cells grown in hemoglobin-containing medium was readily detectable as early as 15 min after incubation with radiolabelled FN and reached equilibrium at 90 min (data not shown).

Similar enhancement of binding activity by hemoglobin was also observed in seven other clinical isolates of *C. albicans* cultured in LBC or YNB medium (Table 1). The enhancement by hemoglobin was consistently greater in YNB medium than in LBC medium. The specificity of enhancement induced by hemoglobin was assessed by an inhibition assay. The enhanced binding activity of *C. albicans* to ^{125}I-FN was inhibited by nonradioactive FN, and this inhibition was dose dependent, with a 50% inhibitory concentration of 31 nM. By Scatchard analysis, the hemoglobin-enhanced FN binding to *Candida* cells was due to interaction with a single class of receptor [$K_d = (2.7 \pm 0.1) \times 10^{-8}$ M; $R = (2.7 \pm 0.4) \times 10^5$ molecules per cell].

On the basis of the results described above, enhanced binding of FN could be due to induction of FN receptor expression by hemoglobin or to a direct effect on FN binding of hemoglobin bound to the surface of blastoconidia. We therefore asked whether the hemoglobin-induced FN binding required growth of *C. albicans* in the presence of hemoglobin. When *Candida* cultures were grown to the stationary phase in hemoglobin-free medium and exposed to hemoglobin at a final concentration of 0.1% for 30 min, their binding activity for ^{125}I-FN was unchanged (data not shown). Similarly, when *Candida*

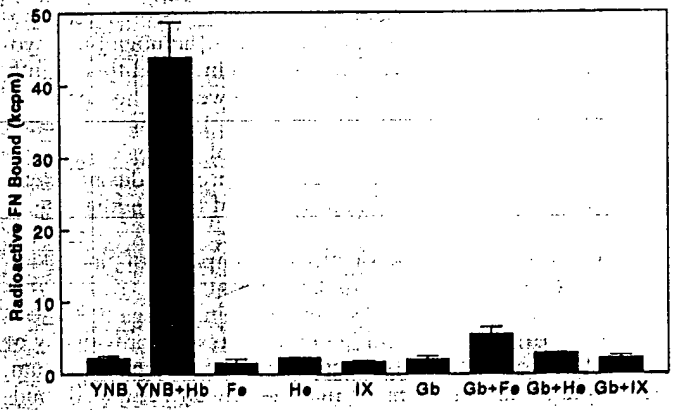

FIG. 3. Specificity of hemoglobin enhancement of FN binding to *C. albicans*. At equivalent molar concentrations of globin subunits or iron, YNB medium was supplemented with hemoglobin (Hb), globin (Gb), hemin (He), ferrous ion (Fe), or protoporphyrin IX (IX) as indicated. *Candida* cells were allowed to grow in these defined media, and their FN binding activity was assessed. Binding data are presented as the means ± standard deviations for three determinations.

TABLE 1. Enhancement by hemoglobin of FN binding activity in *C. albicans* grown in defined media

Strain	Enhancement of FN binding activity[a]	
	LBC + Hb	YNB + Hb
ATCC 44807	5.5 ± 0.3	20.6 ± 6.1
Ca 103	1.6 ± 0.1	3.4 ± 0.3
Ca 126	1.7 ± 0.3	18.8 ± 1.4
Ca 156	2.1 ± 0.1	5.9 ± 0.6
Ca 180	2.1 ± 0.2	10.1 ± 0.7
Ca 195	3.0 ± 1.0	6.5 ± 0.4
Ca 198	1.3 ± 0.3	15.2 ± 0.3
Ca 8621	1.4 ± 0.2	5.3 ± 1.0

[a] Expressed as the fold increase in FN binding relative to that measured for each strain grown without hemoglobin (Hb). In all strains, binding of FN in defined medium without hemoglobin was 1,100 to 1,448 cpm in LBC medium or 984 to 1,613 cpm in YNB medium. Data are expressed as means ± standard deviations.

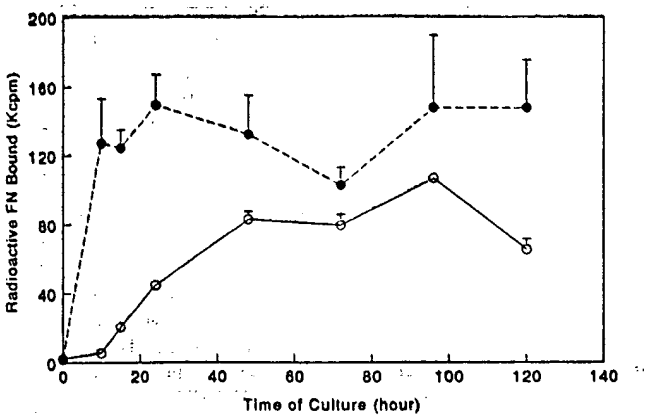

FIG. 4. Time dependence of induction of FN binding. *C. albicans* cells were inoculated in YNB medium in the presence of 0.1% hemoglobin and grown at 26°C (open circle) or 37°C (solid circle) with shaking. At various intervals, 2 × 10^6 blastospores were removed from the culture, washed, and incubated with radiolabelled FN at a final concentration of 0.5 μg/ml for 3 h at room temperature. The radioactivity bound to the cells was quantified as described in Materials and Methods.

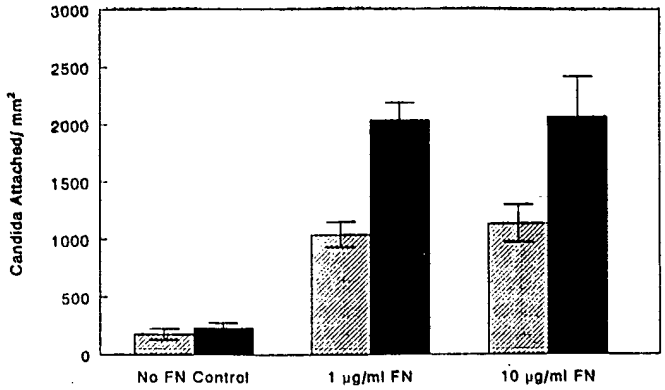

FIG. 5. Growth of *C. albicans* in hemoglobin enhances adhesion to immobilized FN. Chamber Slides were coated with FN at 1 or 10 μg/ml in DPBS by incubation at 4°C overnight, and unbound FN was removed by washing three times with DPBS. *Candida* cells prepared from cultures with (solid bars) or without (hatched bars) hemoglobin in the YNB medium were added to each well at a concentration of 2 × 10^6 CFU/ml and allowed to incubate at room temperature for 2 h; washing three times with DPBS followed. Attached *Candida* cells were fixed, stained, and counted. Aggregates of more than 4 *Candida* cells were not counted. Numbers of cells attached per square millimeter of surface were determined in triplicate and are presented as the means ± standard deviations.

cells grown in hemoglobin-free medium were incubated with ^{125}I-FN in DPBS containing 0.1% hemoglobin for 3 h, FN binding was not enhanced. The acquired enhanced FN binding activity only occurred in *Candida* cells when they were grown in medium containing hemoglobin. The expression of FN binding activity was time dependent in hemoglobin-containing medium (Fig. 4). *Candida* cells grown at 26°C demonstrated an increased FN binding activity 10 h after entering the growth phase, and a peak of binding was reached at 48 h which was sustained as long as 5 days after inoculation. Similar enhancement was achieved much sooner when *Candida* cells were allowed to grow at 37°C.

The hemoglobin-enhanced FN binding to *C. albicans* was persistent with organisms grown in the hemoglobin-containing medium. After growth in medium containing hemoglobin for 20 to 48 h, *Candida* cells were transferred to a separate sterile tube, ensuring that no debris or precipitate was carried over, centrifuged at 3,000 × *g* for 2 min, and suspended in fresh YNB medium to give an equivalent cell density. The cell population was not disturbed except for the removal of hemoglobin in the medium. Because of an inoculum effect (11, 19), further growth of *Candida* cells was minimal. Twenty hours after resuspension in hemoglobin-free medium, FN binding to these transferred *C. albicans* cells persisted at a level similar to that detected immediately after transfer (data not shown). However, if only a fraction (50 to 100 μl) of the hemoglobin-enhanced culture was inoculated into 6 ml of fresh medium without hemoglobin and allowed to grow at room temperature, the high FN binding activity was completely reversed in 12 to 20 h (data not shown).

Hemoglobin-enhanced adhesion of *C. albicans* to immobilized FN or endothelial cells. Growth of *C. albicans* in medium containing hemoglobin enhanced adhesion to immobilized FN (Fig. 5). Chamber Slides were coated with FN at concentrations of 1 or 10 μg/ml. *C. albicans* ATCC 44807 grown with hemoglobin demonstrated a significantly greater adhesion to immobilized FN ($P = 0.014$ by two-tailed t test at 1 μg of FN per ml; $P = 0.05$ at 10 μg of FN per ml) than those grown in YNB medium alone. *Candida* cells prepared from cultures grown in the presence of hemoglobin also demonstrated significantly increased adhesion to the apical surface of bovine corneal endothelial cells compared with those grown in medium without hemoglobin (Fig. 6) ($P = 0.048$). Similar increased adhesion was also observed in a clinical isolate, Ca 8621 (data not shown). Adhesion of *C. albicans* to secreted extracellular matrix after removal of the endothelial cells or to wells without cells, however, was not altered by growth with hemoglobin (Fig. 6) ($P = 0.44$ and 0.3, respectively).

DISCUSSION

We have previously reported that growth of *C. albicans* in Sabouraud broth induces two classes of receptors for FN (27). Induction of this phenotype by complex medium is a function of the brand and lot of culture medium used and has been shown to fluctuate in laboratories (5, 27). Our present results

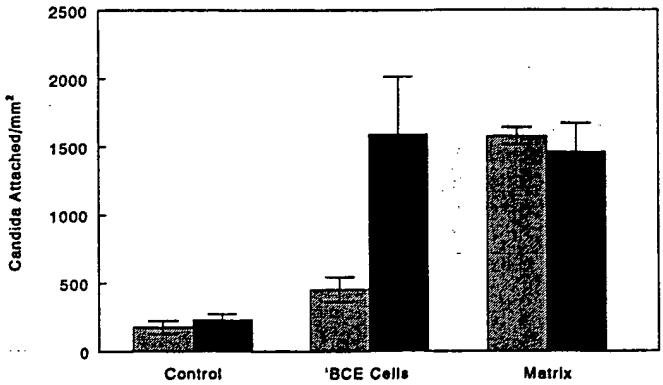

FIG. 6. Growth of *C. albicans* with hemoglobin enhances adhesion to endothelial cells. Bovine corneal endothelial cells were grown to confluence on Chamber Slides as described in Materials and Methods and washed three times with DPBS. A suspension of *Candida* cells prepared from either hemoglobin-enhanced (solid bars) or nonenhanced (hatched bars) cultures in a final concentration of 2 × 10^6 CFU/ml was added to each well, and the Chamber Slides were incubated at room temperature for 2 h. The wells were washed, the cells were fixed and stained, and attached *Candida* cells were counted microscopically. Control represents the background adhesion to slides without endothelial cells. *Candida* cells attached to both supracellular areas and matrix areas where endothelial cells were mechanically peeled. Aggregates of more than 4 *Candida* cells were not counted. The results represent triplicate experiments and are expressed as means ± standard deviations.

demonstrate that high-molecular-weight components of Sabouraud broth, probably derived from the Peptamin component, are responsible for this induction. Although defined media do not induce FN binding, supplementing these media with hemoglobin specifically induced FN binding to *C. albicans*. This induction was consistently observed in a laboratory strain and several clinical isolates of *C. albicans*.

Hemoglobin-induced enhancement of FN binding activity is time dependent and reversible. Acquisition of the phenotype requires the *Candida* cells to grow in the presence of hemoglobin, and the FN binding activity increases as a function of the time after exposure to hemoglobin in a growing status. A detectable increase of FN binding activity was found as early as 5 h after entering the growth phase, and a plateau was reached at 20 h. The hemoglobin-induced phenotype was retained following removal of hemoglobin but was reversed if the cells were regrown in a simple medium without hemoglobin. Failure to induce the binding following preincubation of blastoconidia with hemoglobin or performing the binding assay in the presence of hemoglobin suggests that the increased binding is not simply due to hemoglobin binding to the surface of the yeast and acting as a bridge molecule for binding FN. The small enhancement of FN binding observed with gamma globulins (immunoglobulin G) may result from such a bridging event, since antibodies to cell wall components are probably present and are known to bind FN (32).

Delivery of iron was considered a potential mechanism for induction of FN binding, since both hemoglobin and hemin are able to restore growth of *Candida* cells previously inhibited by transferrin (24). The significance of iron in microbial infection has long been observed (4, 28, 35). Extensive research and clinical observations demonstrate a vigorous competition for essential iron between pathogens, including yeasts, and their vertebrate hosts (9). The ability of microorganisms to acquire iron is an important component of virulence (4, 28, 35). Iron-deprived media limit the growth of *Candida* cells (9, 13), whereas excess iron stimulates growth (8, 9, 12). Clinically, increased iron content of skin may enhance susceptibility to fungal infection (18). These findings correlated well with a recent report that *C. albicans* produced a hemolysin, presumably to allow the *Candida* cells to acquire hemoglobin from host erythrocytes (24).

On the basis of our preliminary results, the hemoglobin-induced enhancement of FN binding activity cannot be explained merely by its ability to provide the essential nutrient iron. Hemin also restores growth (24) but does not induce FN binding. The induction caused by hemoglobin is specific in that other iron-containing proteins, porphyrins, and inorganic iron salts lacked activity. In addition, the weak induction by globin, alone or mixed with heme, suggests that a receptor recognizing the protein may be expressed on *Candida* cells. Myoglobin, a related protein with a physiological function similar to that of hemoglobin but having only 27% sequence identity to hemoglobin, produced a weaker and inconsistent effect, indicating that the native conformation of hemoglobin is specifically recognized. Hemoglobin may deliver iron to the yeast following binding to a specific receptor on the surface or transduce a signal following binding to a receptor. Thus, the production of a hemolytic factor by *C. albicans* (24) may enhance pathogenicity both by providing iron from host erythrocytes and by releasing hemoglobin to enhance FN binding activity. Although the exact mechanism of induction of FN binding activity in *Candida* cells by hemoglobin remains unknown, interactions with host cells were recently demonstrated to alter gene expression and signal transduction in the yeast (2).

The influence of culture media on the activity of antifungal agents (7, 15, 25) and adhesion (5, 34) of *C. albicans* has been well described. The activity of ketoconazole against *C. albicans* grown in Sabouraud glucose medium varied 1,000-fold when the pH of the medium was increased from 3 to 7 (25). Similarly, Hoeprich and Finn described obfuscation of the antifungal activity by six different culture media (15). Of the six conventional media used, only one was a synthetic formulation and the remaining five were complex and undefined. Hoeprich and Finn concluded that meaningful data cannot be obtained from the susceptibility testing of *C. albicans* in vitro unless the potential interaction between antifungal agents and culture medium could be standardized by use of a defined culture medium (15). Our results clearly demonstrate that using hemoglobin as a supplement in a defined medium produced a higher and more stable induction of FN binding activity in *C. albicans* than that produced with complex media or defined, unsupplemented medium. In addition, this medium may assist in further characterizing the induced FN receptor.

A potential role of hemoglobin-induced FN binding activity in the pathogenesis of *C. albicans* is suggested by the increased adhesion of hemoglobin-induced *C. albicans* to immobilized FN and to cultured endothelial cells in vitro. This observation combined with the recent report that *C. albicans* expresses a hemolysin suggests that hemoglobin may serve as an important regulator of adhesive phenotype following entry of the pathogen into the vascular compartment or a wound (16, 24). Although the enhanced adhesion to the endothelial cells was not proven to be mediated by FN (10), the enhancement was specific in that adhesion to extracellular matrix secreted by these endothelial cells, which is rich in laminin and heparan sulfate proteoglycans (26), was not enhanced by hemoglobin. Further investigation will examine the effects of hemoglobin on the interaction of *C. albicans* with other components of the extracellular matrix.

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REFERENCES

1. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 1008. CRC Press, Boca Raton, Fla.
2. **Bailey, A., E. Wadsworth, and R. Calderone.** 1995. Adherence of *Candida albicans* to human buccal epithelial cells: host-induced protein synthesis and signaling events. Infect. Immun. 63:569–572.
3. **Bozzini, S., L. Visa, P. Pignatti, T. E. Petersen, and P. Speziale.** 1992. Multiple binding sites in fibronectin and the staphylococcal fibronectin receptor. Eur. J. Biochem. 207:327–333.
4. **Bullen, J. J.** 1981. The significance of iron in infection. Rev. Infect. Dis. 3:1127–1138.
5. **Calderone, R. A., and P. C. Braun.** 1991. Adherence and receptor relationships of *Candida albicans*. Microbiol. Rev. 55:1–20.
6. **Calderone, R. A., and W. M. Scheld.** 1987. Role of fibronectin in the pathogenesis of candida infections. Rev. Infect. Dis. 9:S400–S403.
7. **Calhoun, D. L., and J. N. Galgiani.** 1984. Analysis of pH and buffer effects on flucytosine activity in broth dilution susceptibility testing of *Candida albicans* in two synthetic media. Antimicrob. Agents Chemother. 26:364–367.
8. **Caroline, L., F. Rosner, and P. J. Kozinn.** 1969. Elevated serum iron, low unbound transferrin and candidiasis in acute leukemia. Blood 34:441–451.
9. **Caroline, L., C. L. Taschdjian, P. J. Kozinn, and A. L. Schade.** 1964. Reversal of serum fungistasis by addition of iron. J. Invest. Dermatol. 42:415–419.
10. **Douglas, L. J.** 1994. Adhesin-receptor interactions in the attachment of *Candida albicans* to host epithelial cells. Can. J. Bot. 73(Suppl. 1):S1147–S1153.
11. **Eagle, H.** 1952. Experimental approach to the problem of treatment failure with penicillin. I. Group A streptococcal infection in mice. Am. J. Med. 13:389–399.
12. **Elin, R. J., and S. M. Wolfe.** 1974. The role of iron in nonspecific resistance to infection induced by endotoxin. J. Immunol. 112:737–745.
13. **Esterly, N. B., S. R. Brummer, and R. G. Crounse.** 1967. The relationship of transferrin and iron to serum inhibition of *Candida albicans*. J. Invest. Dermatol. 49:437–442.

14. **Filler, S. G., J. N. Swerdloff, C. Hobbs, and P. M. Luckett.** 1995. Penetration and damage of endothelial cells by *Candida albicans*. Infect. Immun. **63**:976–983.
15. **Hoeprich, P. D., and F. D. Finn.** 1972. Obfuscation of the activity of antifungal antimicrobics by culture media. J. Infect. Dis. **126**:353–361.
16. **Hostetter, M. K.** 1994. Interaction of *Candida albicans* with eukaryotic cells. ASM News **60**:370–374.
17. **Hostetter, M. K.** 1994. Adhesins and ligands involved in the interaction of *Candida* spp. with epithelial and endothelial surfaces. Clin. Microbiol. Rev. **7**:29–42.
18. **King, R. D., H. A. Khan, J. C. Foye, J. I. Greenberg, and H. E. Jones.** 1965. Transferrin, iron, and dermatophytes. J. Lab. Clin. Med. **86**:204–212.
19. **Kirby, W. M. M.** 1945. Bacteriostatic and lytic actions of penicillin on sensitive and resistant staphylococci. J. Clin. Invest. **24**:165–169.
20. **Klotz, S. A.** 1994. Plasma and extracellular matrix proteins mediate in the fate of *Candida albicans* in the human host. Med. Hypotheses **42**:328–334.
21. **Klotz, S. A., R. C. Hein, R. L. Smith, and J. B. Riuse.** 1994. The fibronectin adhesin of *Candida albicans*. Infect. Immun. **62**:4679–4681.
22. **Klotz, S. A., and R. L. Smith.** 1991. A fibronectin receptor on *Candida albicans* mediates adherence of the fungus to extracellular matrix. J. Infect. Dis. **163**:604–610.
23. **Klotz, S. A., and R. L. Smith.** 1995. Gelatin fragments block adherence of *Candida albicans* to extracellular matrix proteins. Microbiology **141**:2681–2684.
24. **Manns, J. M., D. M. Mosser, and H. R. Buckley.** 1994. Production of a hemolytic factor by *Candida albicans*. Infect. Immun. **62**:5154–5156.
25. **Minagawa, H., K. Kitaura, and N. Nakamizo.** 1983. Effects of pH on the activity of ketoconazole against *Candida albicans*. Antimicrob. Agents Chemother. **23**:105–107.
26. **Munjal, I. D., D. R. Crawford, D. A. Blake, M. D. Sabet, and S. R. Gordon.** 1990. Thrombospondin: biosynthesis, distribution, and changes associated with wound repair in corneal endothelium. Eur. J. Cell Biol. **52**:252–263.
27. **Negre, E., T. Vogel, A. Levanon, R. Guy, T. J. Walsh, and D. D. Roberts.** 1994. The collagen binding domain of fibronectin contains a high affinity binding site for *Candida albicans*. J. Biol. Chem. **269**:22039–22045.
28. **Payne, S. M., and R. A. Finkelstein.** 1978. The critical role of iron in host-bacterial interactions. J. Clin. Invest. **61**:1428–1440.
29. **Pendrak, M. L., and S. A. Klotz.** 1995. Adherence of *Candida albicans* to host cells. FEMS Microbiol. Lett. **129**:103–114.
30. **Penn, C., and S. A. Klotz.** 1994. Binding of plasma fibronectin to *Candida albicans* occurs through the cell binding domain. Microb. Pathog. **17**:387–393.
31. **Roberts, D. D., C. N. Rao, J. L. Magnani, S. L. Spitalnik, L. A. Liotta, and V. Ginsburg.** 1985. Laminin binds specifically to sulfated glycolipids. Proc. Natl. Acad. Sci. USA **82**:1306–1310.
32. **Rostagno, A. A., B. Franglone, and L. Gold.** 1991. Biochemical studies on the interaction of fibronectin with Ig. J. Immunol. **146**:2687–2693.
33. **Santoni, G., A. Gismondi, J. H. Liu, A. Punturieri, A. Santoni, L. Frati, M. Piccolo, and J. Y. Djeu.** 1994. *Candida albicans* expresses a fibronectin receptor antigenically related to alpha-5 beta-1 integrin. Microbiology **140**: 2971–2979.
34. **Sela, S., A. Aviv, A. Tovi, I. Burstein, M. G. Caparon, and E. Hanski.** 1993. Protein F: an adhesion of Streptococcus pyogenes binds fibronectin via distinct domains. Mol. Microbiol. **10**:1049–1055.
35. **Weinberg, E. D.** 1978. Iron and infection. Microbiol. Rev. **42**:45–66.
36. **Yamada, K. M.** 1991. Adhesive recognition sequences. J. Biol. Chem. **266**: 12809–12812.

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